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LABORATORY METHODS USED TO EVALUATE CANDIDATE INSECTICIDES,  
ACARICIDES, ATTRACTANTS, REPELLENTS, AND CHEMOSTERILANTS 4/10

Agricultural Research Service,

Entomology Research Division,  
Market Quality Research Division,  
Plant Pest Control Division,

5  
AC Compiled by the Biological Investigations Unit,  
U.S.A.R.S. Pesticide Chemicals Research Branch, Entomology  
Research Division. (—)

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## Foreword

This revision contains the current laboratory test methods used in preliminary evaluations of candidate insecticides, acaricides, attractants, repellents, and chemosterilants of synthetic or natural origin received from commercial companies, other governmental agencies, universities, and those prepared by the Pesticide Chemicals Research Branch, Entomology Research Division. These evaluations are conducted at 23 laboratories in the Entomology Research Division and one laboratory each in the Agricultural Marketing Research and the Plant Pest Control Divisions. Sixty-five insect species representing eight orders and eight species of Acarina are used in these evaluations.

Descriptions of the evaluation methods are arranged alphabetically by the scientific name of the test species and grouped by orders.

U. S. DEPT.  
ENTOMOLOGY  
RESEARCH DIVISION

FEB 27 1973

GRANT

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PLANT PEST CONTROL DIVISION

Methods Improvement Laboratory  
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ENTOMOLOGY RESEARCH DIVISION - Test Species

Insecticides and Acaricides

<u>Species</u>	<u>Research Branch Conducting Test</u>	<u>Test Method Described on Page</u>
<u>Acarina</u>		
<u>Aculus pelekassi</u> a rust mite	FV	9
<u>Amblyomma americanum</u> lone star tick	MA	10
<u>Eutrombicula alfreddugesi</u> a chigger	MA	10
<u>Panonychus ulmi</u> European red mite	FV	10-11
<u>Phyllocoptes oleivora</u> citrus rust mite	FV	11
<u>Tetranychus cinnabarinus</u> carmine spider mite	FV, CI	11
<u>Tetranychus telarius</u> two-spotted spider mite	FV, PC	12-13
<u>Typhlodromus fallacis</u> a predaceous mite	FV	13
<u>Orthoptera</u>		
<u>Blatta orientalis</u> oriental cockroach	PC	14
<u>Blattella germanica</u> German cockroach	MA, PC	14-16
<u>Melanoplus bivittatus</u> two-striped grasshopper	GF	16
<u>Melanoplus sanguinipes</u> migratory grasshopper	GF	16
<u>Periplaneta americana</u> American cockroach	PC	17
<u>Periplaneta brunnea</u> Brown cockroach	PC	17
<u>Supella supellectilium</u> brown-banded roach	PC	17
<u>Anoplura</u>		
<u>Pediculus humanus humanus</u> body louse	MA	17-19
<u>Hemiptera (Heteroptera)</u>		
<u>Cimex lectularius</u> bed bug	MA	19
<u>Lygus hesperus</u>	CI	19

Hemiptera (Homoptera)

<u>Aonidiella aurantii</u>		
California red scale	FV	20
<u>Aphis craccivora</u>		
cowpea aphid	GF	20
<u>Aphis gossypii</u>		
cotton or melon aphid	CI	20
<u>Aphis pomi</u>		
apple aphid	FV	20-21
<u>Myzus persicae</u>		
green peach aphid	FV	21
<u>Psylla pyricola</u>		
pear psylla	FV	21
<u>Rhopalosiphum maidis</u>		
corn leaf aphid	GF	21
<u>Schizaphis graminum</u>		
greenbug	GF	21-22

Lepidoptera

<u>Alabama argillacea</u>		
cotton leafworm	CI	22
<u>Argyrotaenia velutinana</u>		
red-banded leaf roller	FV	22-23
<u>Bucculatrix thurberiella</u>		
Cotton leaf-perforator	CI	23
<u>Carpocapsa pomonella</u>		
codling moth	FV	23-24
<u>Estigmene acrea</u>		
salt-marsh caterpillar	CI	24
<u>Heliothis virescens</u>		
tobacco budworm	CI	24
<u>Heliothis zea</u>		
bollworm or corn earworm	CI	24-25
<u>Laphygma frugiperda</u>		
fall armyworm	GF	25
<u>Ostrinia nubilalis</u>		
European corn borer	GF	25-26
<u>Pectinophora gossypiella</u>		
pink bollworm	CI	26
<u>Prodenia eridania</u>		
southern armyworm	PC	26
<u>Spodoptera exigua</u>		
beet armyworm	CI	27
<u>Trichoplusia ni</u>		
cabbage looper	CI, FV	27

Coleoptera

<u>Anthophonus grandis</u>		
boll weevil	CI, PC	27-28
<u>Conoderus falli</u>		
southern potato wireworm	FV	28
<u>Diabrotica balteata</u>		
banded cucumber beetle	FV	28
<u>Hypera postica</u>		
alfalfa weevil	GF	29

Diptera

<u>Aedes aegypti</u>		
yellow-fever mosquito	MA	29
<u>Aedes sollicitans</u>		
salt-marsh mosquito	MA	30
<u>Aedes taeniorhynchus</u>		
black salt-marsh mosquito	MA	30
<u>Anastrepha ludens</u>		
Mexican fruit fly	FV	30
<u>Anopheles quadrimaculatus</u>		
common malaria mosquito	MA	31
<u>Ceratitidis capitata</u>		
Mediterranean fruit fly	FV	31-33
<u>Cochliomyia hominivorax</u>		
screw-worm	MA	33
<u>Cochliomyia macellaria</u>		
secondary screw-worm	MA	33-34
<u>Culex tarsalis</u>		
	MA	34
<u>Dacus cucurbitae</u>		
melon fly	FV	34
<u>Dacus dorsalis</u>		
oriental fruit fly	FV	35
<u>Drosophila melanogaster</u>		
	FV, GF	35
<u>Gasterophilus</u> spp.		
bot flies	MA	36
<u>Haematobia irritans</u>		
horn fly	MA	36
<u>Hypoderma bovis</u>		
northern cattle grub	MA	36-37
<u>Hypoderma lineatum</u>		
common cattle grub	MA	37
<u>Liriomyza</u> sp. nr. <u>commeliniae</u>		
a leaf miner	FV	37
<u>Musca autumnalis</u>		
face fly	PC	37-38
<u>Musca domestica</u>		
house fly	MA, PC	38-39
<u>Oestrus ovis</u>		
sheep bot fly	MA	39

Diptera (cont.)

<u>Phormia regina</u> black blow fly	MA	39
<u>Stomoxys calcitrans</u> stable fly	MA	39-40

Siphonaptera

<u>Xenopsylla cheopis</u> oriental rat flea	MA	40-42
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Attractants

Orthoptera

<u>Blattella germanica</u> German cockroach	MA	42
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Diptera

<u>Anastrepha ludens</u> Mexican fruit fly	FV	43-44
<u>Ceratitis capitata</u> Mediterranean fruit fly	FV	44-46
<u>Dacus cucurbitae</u> melon fly	FV	46
<u>Dacus dorsalis</u> oriental fruit fly	FV	46
<u>Drosophila melanogaster</u>	FV	46

Repellents

Acarina

<u>Amblyomma americanum</u> lone star tick	MA	46-47
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Orthoptera

<u>Blattella germanica</u> German cockroach	MA, PC	47
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Hymenoptera

<u>Apis mellifera</u> honey bee	A	48
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Diptera

<u>Aedes aegypti</u> yellow-fever mosquito	MA	48
<u>Musca autumnalis</u> face fly	PC	49
<u>Stomoxys calcitrans</u> stable fly	MA	49-50

Chemosterilants

Acarina

<u>Tetranychus telarius</u> two-spotted spider mite	PC	50
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Diptera

<u>Anastrepha ludens</u> Mexican fruit fly	FV	50
<u>Cochliomyia hominivorax</u> screw-worm	MA	51
<u>Musca domestica</u> house fly	MA	51

MARKET QUALITY RESEARCH DIVISION - Test Species

Insecticides

Lepidoptera

<u>Plodia interpunctella</u> Indian meal moth	SP	52
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Coleoptera

<u>Attagenus piceus</u> black carpet beetle	SP	52-53
<u>Tribolium confusum</u> confused flour beetle	SP	54-55

Repellents

Coleoptera

<u>Tribolium castaneum</u> red flour beetle	SP	55
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PLANT PEST CONTROL DIVISION - Test Species

Insecticides

Coleoptera

Graphognathus spp.  
white-fringed beetle 56

Hymenoptera

Solenopsis saevissima  
imported fire ant 56-59

1/ A - Apiculture Research Branch

FV - Fruit and Vegetable Insects Research Branch

MA - Insects Affecting Man and Animals Research Branch

CI - Cotton Insects Research Branch

PC - Pesticide Chemicals Research Branch

GF - Grain and Forage Insects Research Branch

SP - Stored Product Insects Branch

ENTOMOLOGY RESEARCH DIVISION

Test Methods - Insecticides and Acaricides

Acarina

Aculus pelekassi - rust mite

Orlando, Florida

Dip Test:

Aculus pelekassi are reared on Murcott Honey orange seedlings in air conditioned greenhouses at temperatures ranging from 74-84° F. Mites are transferred to clean seedlings by cutting infested leaves in narrow strips and placing them on clean leaves. Lanolin is applied to the base of the leaves to prevent migration of the mites. The infested seedlings are dipped in acetone suspensions of the experimental chemicals within 24 hours after infestation.

For tests at 20 ppm, 16 milligrams of each experimental materials are dissolved in 5 ml. of acetone and suspended in 800 ml. of distilled water. This mixute is continuously agitated in a plastic lined ice cream carton, using a magnetic stirrer. Infested seedlings are inverted, clamped to the arm of a dipping apparatus and submerged in the mixture for 6 seconds. Following treatment, the seedlings are kept in a fume hood until dry and then moved to an air-conditioned greenhouse. Lower concentrations are made up by diluting from a 20 ppm. stock solution.

Mortality of rust mites are estimated 72 hours after treatment.

Standard acaricides in technical grade samples such as Kelthane, dioxathion, and ethion give mortalities ranging from 76 to 99 percent at concentrations of 5 p.p.m. and 100 percent at 20 p.p.m. against both Phyllocoptruta oleivora and Aculus pelekassi. Carbophenothon and chlorobenzilate give similar mortalities in tests against P.oleivora but these materials are not as effective against A. pelekassi.

Amblyomma americanum - lone star tick

Kerrville, Texas

Systemic Test:

Candidate acaricides are screened for systemic activity by treating guinea pigs infested with engorging nymphal lone star ticks. The acaricides, formulated as 5-percent solutions in Tween-20, are administered orally and subcutaneously to two guinea pigs. If ticks engorge normally, they are held until they molt to see whether the candidate material is effective during the molting period. If the guinea pig or ticks are killed at the initial dosage (usually 100 mg./kg.), lower dosages are tested until there is no acaricidal activity and/or no toxicity to the guinea pig.

Eutrombicula alfreddugesi - a chigger

Gainesville, Florida

Patch Test:

Cotton twill patches, 4 inches square, are impregnated with 0.222 grams (2.0 gm./sq.ft.) of the material to be tested. The patches are placed on glass plates, a rubber fruit-jar ring is placed on the cloth, and five chiggers are confined in the ring by covering it with another glass plate. If effective (all chiggers down in 15 minutes or less), the treated cloth is rinsed in cool water for 15 minutes and retested. If still effective a fresh sample is subjected to repeated 10-minute washes in hot soapy water, each followed by a 10-minute cool water rinse, and tested after each wash and rinse until less than 100% knockdown is achieved within the 15-minute exposure period. Under these conditions, the standard chigger toxicant, benzyl benzoate, withstands two or more washes.

Panonychus ulmi - European red mite

Wenatchee, Washington

Spray Test:

Fruit trees, usually peach seedlings, grown in pots are sprayed with varying concentration of emulsions, solutions, or suspension of the candidate acarcide while being rotated on a turntable. After the sprays dry, disks are cut from selected leaves with a cork borer and placed with the upper side down on slightly larger disks of white blotting paper. These disks, usually 10 for each treatment, are arranged on cellulose-sponge pads in petri dishes partly filled with distilled water.

Each disk is then infested with adult mites. The dishes are left uncovered and distilled water is added daily to maintain the water barrier as a deterrent to migration of the mites. Examinations are made after 3 days and again after 1 week to record adult mortality and the number of eggs laid. In some tests samples of leaves are brushed with a special machine to dislodge the mites for counting. Kelthane is used as the standard.

Phyllocoptruta oleivora = citrus rust mite

Orlando, Florida

Dip Test:

Method is the same as described for *Aculus pelekassi* (see page 9).

Tetranychus cinnabarinus = carmine spider mite

Beltsville, Maryland

Dip Test:

Lima bean seedlings in the two-leaf stage in bottles of nutrient solution are dipped in the candidate acaricide at dosages of 2 to 16 ounces of active toxicant per 100 gallons. One set of plants bears spider mites transferred 24 hours previous to treatment and supplies information on contact action. Four additional sets of plants are infested with mites at 0, 1, 3, and 7 days, respectively, after treatment. Contact and residual activity is determined by counts under the binocular microscope 1 and 3 days after initial exposure.

College Station, Texas

Systemic Test:

The technical candidate acaricide is dissolved in an organic solvent and mixed with agricultural-grade carbon to make a 50-percent carbon dust (w/w). Cotton seeds are treated at the rate of 4 pounds of active ingredient per 100 pounds of seed (equivalent to 1 pound per acre). Methyl cellulose is added as a sticker. The seeds, methyl cellulose, and carbon-acaricide mixture are placed in a jar and mechanically rolled for 30 minutes. The seeds are then planted in 1-gallon cans of soil, 6 seeds per can. Upon emergence the cotton plants are tested for acaricidal activity by placing spider mites on a terminal leaf of a treated plant. Mortality is recorded after 3 days. Tests are continued until the plants are no longer toxic to the spider mites. Phorate is used as the standard.

Tetranychus telarius - two-spotted spider mite

Brownsville, Texas

Spray Test:

Cotton seedlings infested with spider mites (20 or more per plant) are exposed, on a turntable in a wind tunnel, to sprays of 5 ml. of an acetone solution of the candidate acaricide. Various concentrations up to 0.25 percent are used to give a range of kills. Each treatment is replicated 4 times. Mortality is recorded after 72 hours. Malathion is used as the standard.

Systemic Test:

Cotton seedlings are placed in plant nutrient solution containing various concentrations of the candidate acaricide. Three days later the seedlings are infested with spider mites (20 or more per plant). Each treatment is replicated 4 times. Mortality is recorded 48 hours after the seedlings are infested. Demeton is used as the standard.

Residual Test:

Cotton seedlings in individual pots are placed on a turntable in a wind tunnel and exposed to sprays of 5 ml. of an acetone solution of the candidate material. Treated plants are weathered in a screen house having partial shade. Plants are infested with spider mites at the following intervals: as soon as residue is dry, 3 days after spraying, 7 days after spraying, and 14 days after spraying.

Mortality is recorded 72 hours after the spider mites are introduced onto treated plants. Trithion is used as the standard.

Vincennes, Indiana

Spray Test:

Candidate acaricides are applied as sprays at the rate of 2 ounces of toxicant per 100 gallons to potted, two-leafed, lima bean plants which have been infested with five adult mites per leaf 4 days before treatment. Four infested plants per treatment are sprayed on a turntable sprayer. Adults, nymphs, and eggs are counted on one leaf of each plant 3 days after treatment and on the other leaf at 7 days. Composite samples of mites on the bean plants are prepared by brushing them into a binder on a glass plate with a special brush-machine. Water-sprayed, infested plants are used as controls.

Systemic Test:

The method is the same as described for the spray test except the candidate acaricide is injected with a hypodermic syringe into the sand in the pots containing bean plants.

Wenatchee, Washington

Spray Test:

Method is the same as described for the European red mite (see page 10).

Beltsville, Maryland

Dip Test:

Tests are conducted to find acaricides showing contact and residual activity against one to three highly resistant strains of the two-spotted spider mite; a parathion resistant (Cranbury-1), a Kelthane-resistant (Cranbury-10) or a super-resistant (Hart mite) strain. A non-resistant strain (Mass.) is used as the standard. Method is the same as described for the carmine spider mite (see page 11).

Typhlodromus fallacis - a predaceous mite

Beltsville, Maryland

Dip Test:

Residual toxicity of commercially available fungicides, insecticides, and acaricides is tested against this predaceous mite in a search for materials that can be applied to crops for pest control in an integrated chemical-biological control program.

Lima bean seedlings are dipped in emulsions or suspensions of the test chemical at standard dosages as described for the carmine spider mite, (page 11). As soon as the foliage has dried, the plants are infested with Cranbury-10 or Hart resistant strains of the two-spotted spider mite which tolerate the residues of all but one or two known acaricides. Upon becoming established on the treated plants, they provide freshly laid eggs and individuals in other stages as food for the predaceous mites. Twenty-four hours after treatment, five female predator mites are transferred by brush to each of four two-spotted spider mite-infested plants. Observations on mortality of predators are made 24 and 72 hours after transfer. If the predators are killed, additional sets of five adults are transferred to the treated plants and the observations repeated until the toxic effect of the residue has subsided. Chemicals with residues showing no or short residual activity are considered favorable for the integrated program.

Orthoptera

Blatta orientalis - oriental cockroach

Beltsville, Maryland

Residue Test:

Acetone solutions of the candidate insecticides are applied to the inner surfaces of glass fruit jars. Jars are treated in duplicate with each concentration of the insecticide by rolling the solution evenly over the bottom and sides (up to the neck) until the acetone dries. Male adult cockroaches are then placed in each jar. Mortality is recorded hourly for the first 8 hours in some tests and 24, 48, and 72 hours in all tests. All candidates superior to the standard are held for aging studies and tested again after 1 week, 2 weeks, 4 weeks, and until 100 percent mortality is no longer reached after 24 hours exposure. Chlordane is used as the standard.

Blattella germanica - German cockroach

Gainesville, Florida

Contact Spray Test:

In contact spray tests with cockroach toxicants, 0.5 ml. of a 2.0-percent solution of the compound in deodorized kerosene is sprayed into a wind tunnel. When necessary, acetone or other auxilliary solvents are used to assure complete solution of the compounds. Twenty adult male cockroaches in screen-wire cages (2 replicates of 10 each) are exposed momentarily to the spray as it is drawn through the tunnel by a 8-m.p.h. draft, after which the cockroaches are transferred to clean petri dishes. Knockdown and kill are recorded after 10, 30, and 60 minutes and 24 hours. If 75- to 100-percent knockdown and kill are produced within 24 hours after exposure, the compound is then tested at 0.5-percent concentration. All tests are conducted in comparison with a chlordane standard.

Residue Test:

Compounds that produce 75- to 100-percent knockdown and kill within 24 hours as a 2.0-percent contact spray are tested as residues on plywood panels. The panels are treated, by means of a calibrated spraying machine, with an acetone solution containing 1.4 percent of toxicant to produce a residue of 100 milligrams per square foot. When warranted, water suspensions and emulsions are substituted for the acetone solutions.

The treated panels are allowed to dry for 2 hours, then 20 adult male cockroaches (2 replicates of 10 each) are exposed to the residues for 30 minutes. Exposures to the treated surfaces are made under inverted plastic dishes coated on the inner surface with pyrophyllite, which prevents the cockroaches from crawling up the sides. At the conclusion of the exposure period, the cockroaches are transferred to clean petri dishes. Observations on knockdown and kill are made at this time and after 24 and 48 hours. The treated panels are then allowed to age and tested at intervals of 1, 2, and 4 weeks, or longer if necessary, depending on the effectiveness of the residues. All tests are conducted in comparison with a chlordane standard.

Dust Test:

Dust are applied to 5-1/4 inch square plywood panels in a dust tower which consists of a bell jar 14 inches high with an inside diameter of 8-1/4 inches. An inverted glass funnel is inserted from the inside of the bell jar through a one-hole stopper; the stem of the funnel projects 4 inches above the jar. A small rubber stopper tied to the outside of the glass funnel is placed inside the cone to retain the dusts in the stem until ejection by compressed carbon dioxide.

A 15-ml., volumetric, transfer pipette, with both ends severed 2 cm. from the bulb, is cut across the bulb to provide two sections of unequal volume. The dust is weighed in the larger section. The two sections are rejoined with masking tape and one end is connected to the protruding funnel stem by a 1-inch piece of rubber tubing, and the other end is connected to a source of carbon dioxide under a pressure of 20 to 30 p.s.i., which is turned on for 0.4-0.6 second to expel the dust.

The interior shape of the dust chamber is modified by the addition of a glazed-paper cone which extends from the funnel to the bottom perimeter of the bell jar. After ejection of the dust, the panel remains under the tower for 3 minutes, then the bell jar is cautiously removed to minimize air movement over the dusted surface. A plastic dish, 3-1/2 inches in diameter, with a small opening in the bottom, is coated with pyrophyllite and inverted over the panel. The pyrophyllite coating forces the cockroaches to remain in contact with the treatment, since they cannot crawl on pyrophyllite-coated plastic.

Ten male cockroaches are dropped through the dish opening onto the dusted surface. After exposure for 30 minutes, they are removed and placed in a petri dish. Mortality counts are taken 24 and 48 hours later. The temperature in the test room is maintained at 80° F. to 84° F. throughout the test.

Beltsville, Maryland

Residue Test:

Method is the same as described for the oriental cockroach (see page 14). Chlordane is used as the standard for susceptible cockroaches and malathion for resistant ones.

Melanoplus bivittatus - two-striped grasshopper

Bozeman, Montana

Topical Test:

The technical candidate insecticide dissolved in a solvent, usually acetone, is applied to the first two abdominal sternites of adult grasshoppers or fifth-instar nymphs or to lettuce discs for oral tests. Applications are made with a tuberculin syringe fitted with a hypodermic needle and driven by a micrometer screw.

Dosage levels tested are compared with those produced by recrystallized aldrin at the LD-50 level. After topical treatment the grasshoppers are caged with untreated lettuce as food. For the oral test they are fed on the treated discs. Mortalities are recorded at 24, 48, and 72 hours.

Spray Test:

Grasshoppers and/or potted wheat plants are sprayed with emulsions, suspensions, or solutions of the candidate insecticide. The sprayer consists of an open sheet-metal drum mounted on a belt-driven turntable and revolved within a hood equipped with an air-exhaust system. A DeVilbiss atomizer nozzle, adjustable vertically, is mounted above the center of the spray chamber. Amounts of spray as low as 1 gallon per acre can be obtained while the drum makes several revolutions. Grasshoppers alone, grasshoppers and potted wheat plants, or potted wheat plants alone are sprayed. Grasshoppers treated alone are caged with untreated lettuce as food. Wheat plants treated are subjected to feeding by treated or untreated hoppers. Mortality is recorded at 24, 48, and 72 hours. Recrystallized aldrin, 2 ounces in 1 gallon of solution per acre, is used as the standard.

Melanoplus sanguinipes - migratory grasshopper

Bozeman, Montana

Methods are the same as described for the two-striped grasshopper (See above).

Periplaneta americana - American cockroach

Beltsville, Maryland

Residue Test:

Method is the same as described for the oriental cockroach.  
(See page 14).

Periplaneta brunnea - brown cockroach

Beltsville, Maryland

Residue Test:

Method is the same as described for the oriental cockroach.  
(See page 14).

Supella supellectilium - brown-banded roach

Beltsville, Maryland

Residue Test:

Method is the same as described for the oriental cockroach.  
(See page 14).

Anoplura

Pediculus humanus humanus - body louse

Gainesville, Florida

Beaker Test:

Compounds are screened as louse toxicants by exposing young adult body lice on treated patches of woolen cloth. The patches are dipped in 1-percent solutions of the compounds in acetone or another volatile solvent and impaled on pin points to dry. Lice are exposed on the patches in glass beakers for 24 hrs. Knockdown is recorded at intervals of 15 minutes, 1 hour, and 3 hours, and kill at 24 hours. Patches on which all lice are dead or knocked down are retested at intervals of 2 to 7 days until one or more lice remain unaffected. After 31 days the tests are terminated even if the patches are still effective. DDT, the standard louse toxicant, is effective for more than 31 days under these conditions.

Classification of effectiveness:

1. Ineffective (one or more lice survived initial exposure to freshly treated pad).
2. Effective (no survivors) on the initial exposure but ineffective on the second exposure started 1 day after treatment.
3. Effective on the second exposure but failed on or before the tenth day.
4. Effective for 10 days or more.
- 4A. Effective for 31 days or more.

Classification of knockdown:

1. Incomplete in 24 hours.
2. Complete in 24 hours but not in 3 hours.
3. Complete in 3 hours but not in 1 hour.
4. Complete within 1 hour.
- 4A. Complete within 15 minutes.

Powder Panel Tests:

In powder panel tests, 0.5 gram of a 1-percent powder of the candidate compound, prepared by mixing an acetone solution with pyrophyllite and evaporating the acetone, is spread on a balbriggan cloth patch 4.9 inches square (1/6 sq. ft.) held by thumbtacks to a plywood panel. Twenty adult lice are exposed on these patches for 24 hours, confined by large-mouth mason jar rings, without covers. The patches are tested when fresh and at intervals thereafter until some of the lice remain normal after a 24-hour exposure. Other tests are conducted with 5-percent powders of the toxicants that are 90-100 percent effective as fresh treatments when tested as 1-percent powders but have a short period of residual effectiveness. Both lindane and DDT are tested concurrently with the candidate toxicants for comparison.

Sleeve Test:

In sleeve tests, cloth sleeves (1 sq. ft.) are treated with 3 grams of powders containing the candidate insecticides at various concentrations. The sleeves are worn on the arms or legs of human subjects and adult body lice are periodically introduced, with observations of mortality after 24 hours, so that a measure of residual effectiveness may be obtained.

Systemic Test:

Preliminary evaluations of compounds as systemic insecticides for the control of the body louse are made by administering the insecticide orally to rabbits and feeding the lice on the treated rabbits.

Various dosages of the materials in solution or suspension are administered by means of a stomach tube. Lots of 20 young female lice are fed on the rabbits at hourly intervals up to 5 hours after treatment. After feeding, the lice are kept on wool cloth pads in beakers at 80° F. and 60 to 70 percent relative humidity. The lice are examined for mortality after 24 hours.

Hemiptera (Heteroptera)

Cimex lectularius - bed bug

Gainesville, Florida

Residue Test:

Circular pieces of Whatman No. 1 and 2 filter paper 1.5 inches in diameter, are impregnated with 0.2 ml. of an acetone solution containing 0.31 percent of an insecticide. This impregnation rate provides an equivalent of 50 mg. of toxicant per square foot. After drying for 1 hour, each treated paper is placed in a 50-ml. beaker and ten adult bugs that have been starved for 3 days are placed thereon. After exposure for 24 hours, live and affected bed bugs are removed from the treated paper and placed on untreated filter paper in a clean 50-ml. beaker for a holding period of 24 hours, after which mortality readings are made. The treated papers are aged in the beakers and tested at various intervals of time.

Lygus hesperus

Tucson, Arizona

Spray Test:

Varying concentrations of solutions or emulsions of the candidate insecticide are tested at dosages calculated in pounds per acre. The starting dosages are 1 pound for organophosphorus candidates and 2 pounds for chlorinated hydrocarbon and carbamate candidates. Dosages are reduced until kills are less than 100 percent. Greenhouse-grown cotton plants are sprayed in a fume hood. Immediately after spraying, they are placed in a holding room, cages placed over them, and 10 to 20 two-day-old adult lygus bugs introduced into each cage. Mortality is recorded 24, 48, and 72 hours after treatment. The standard insecticides are a 3:1 toxaphene-DDT mixture and Zectran.

Aonidella aurantii - California red scale

Riverside, California

Spray Test:

Lemons infested with scales of uniform age are sprayed soon after the scales have begun to reproduce. The sprayed lemons are held in a room at 80° F. until mortality counts are made about 6 weeks later. During the holding period, scales of the new generation have had a chance to settle and grow, except as prevented by the spray residue. If inspection indicates any significant residual action when mortality records are made, all of the old scales are removed and the number of new-generation scales that become adult is determined to give a measure of residual effectiveness. Emulsions, solutions and wettable powders applied with DeVilbiss sprayer. One concentration with six replicates with count of 50 scales per replicate (each lemon) are used in the initial screening. Parathion is the standard.

Aphis craccivora - cowpea aphid

Tifton, Georgia

Systemic Tests:

Candidate insecticides prepared in serial dilutions are injected with a syringe into the soil of potted lupine plants infested with aphids. Phorate is used as a standard and untreated potted plants as controls. Results are based upon time and degree of mortality occurring among the treatments. Treatments are replicated twice.

Aphis gossypii - cotton or melon aphid

College Station, Texas

Systemic Test:

Method is the same as described for the carmine spider mite. (See page 11).

Aphis pomi - apple aphid

Wenatchee, Washington

Spray Test:

Potted pear seedlings are sprayed with varying concentrations of solutions, emulsions, or suspensions of the candidate insecticide with an atomizer while rotating on a turntable. After the sprays

have dried, discs are cut from selected leaves. The discs are placed on discs of white blotting paper of the same size, with the under surface up. They are then placed on slightly larger discs of cellulose sponge material about 1/3-inch thick. This assembly is then tightly wedged into a clear plastic ring to form the bottom of a cage. Aphids are placed on the leaf surface and a fine wire screen used for a top. These cages are then placed in culture dishes of distilled water to maintain plant vigor during the test period. Mortality is recorded after 24 and 48 hours. Parathion is used as the standard insecticide.

Myzus persicae - green peach aphid

Wenatchee, Washington

Spray Test:

Method is the same as described for the apple aphid. (See page 20).

Psylla pyricola - pear psylla

Wenatchee, Washington

Spray Test:

Method is the same as described for the apple aphid. (See page 20).

Rhopalosiphum maidis - corn leaf aphid

Stillwater, Oklahoma

Spray Test:

Emulsions, solutions, or suspensions of the candidate insecticide are applied to potted barley plants infested with aphids. The sprays are applied in a cylindrical glass settling chamber containing a turntable. Three replications of infested plants are used for each concentration of the insecticide. Concentration equivalents of 0.063, 0.032, and 0.016 pound per acre are used. Mortality is determined at 3, 24, and 48 hours after treatment. Parathion is used as the standard. If complete mortality is obtained, the plants are reinfested to determine residual toxicity.

Schizaphis graminum - greenbug

Stillwater, Oklahoma

Spray Test:

Method is the same as described for the corn leaf aphid. (See above).

Granular Formulation Test:

Soil in greenhouse flats is treated with granular formulations of candidate insecticides at starting dosages of 0.125 and 0.25 pounds actual toxicant per acre. The flats contain wheat or barley plants which are artificially infested with greenbugs. The aphids on the plants in the two center rows in the flat are counted. Three replications of infested plants are used for each concentration of the insecticide. Mortality is determined at 3, 24, and 48 hours after treatment. If complete mortality is obtained, the plants are reinfested to determine residual toxicity. Di-Syston is used as the standard.

Lepidoptera

Alabama argillacea - cotton leafworm

Brownsville, Texas

Spray Test:

Candidate insecticides are screened by exposing larvae to spray deposits on cotton plants. Potted plants are placed on a turn-table in a spray chamber and sprayed with a measured amount of an emulsion or suspension containing the technical material at a calculated application rate per acre. After the spray deposit has dried on the treated plants a laboratory cage is placed over each plant and 10 or 20 nearly full-grown larvae per plant are placed on the caged plants. Larval mortalities are recorded after 24 and 48 hours. Methyl parathion is used as the standard.

Argyrotaenia velutinana - red-banded leaf roller

Vincennes, Indiana

Larvicide Test:

Candidate insecticides are applied as solution, emulsion, or suspension sprays to potted lima bean plants on a turntable in a ventilated booth. After the spray has dried, two primary leaves on each plant are folded over and fastened with a staple to form a hiding place for the larvae. Two larvae are then placed on each leaf. Each treatment is replicated five times, with a total of 20 larvae per treatment. Mortality is recorded on the fourth day after the larvae are placed on the leaves. Water-sprayed plants are used as controls.

Adulticide Test:

Untreated apple terminals with 10 to 15 intact leaves are dipped in emulsions, solutions, or suspensions containing 2, 4, and 8 ounces (actual toxicant) of organophosphorus candidate insecticides or twice these concentrations of other candidate insecticides.

After the dipped leaves have dried for 24 hours, they are removed from the terminal and 20 leaves are placed in a 2-pound plastic cottage-cheese can, containing moist sand in the bottom. Ten adults are placed in the can through a hole in the clear plastic lid. Two cans (20 moths) are used for each treatment. Cans containing moths are placed in a lighted holding room. Mortality is recorded at 24, 48, and 72 hours after adult introduction. Water-dipped leaves are used as controls. Dipped corrugated paper bands, cut to fit inside the circumference of the cans are used in place of apple leaves during the dormant period of the apple trees when leaves are not available.

Bucculatrix thurberiella - cotton leaf perforator

Tucson, Arizona

Spray Test:

Varying concentrations of solutions or emulsions of the candidate insecticide are tested at dosages calculated in pounds per acre. The starting dosages are 1 pound of organophosphorus candidates and 2 pounds for chlorinated hydrocarbon and carbamate materials. Dosages are reduced until kills are less than 100 percent. Greenhouse-grown cotton plants are sprayed in a fume hood. Immediately after spraying, they are placed in a holding room, cages placed over them, and fourth- and fifth-instar larvae introduced into the cages. Mortality is recorded at 24, 48, and 72 hours after treatment. Endrin or Sevin is used as the standard.

Carpocapsa pomonella - codling moth

Vincennes, Indiana

Ovicide Test:

Pear sticks containing eggs are sprayed with emulsions, solutions, or suspensions of the candidate insecticide on the turntable in a ventilated booth. After spraying, the sticks are placed on racks in a holding room and held for 6 days, at which time mortality is determined by counting hatched and unhatched eggs. Water-sprayed checks are used in each test.

Larvicide Test:

Emulsion, solution, or suspension sprays of the candidate insecticide at varying concentrations are applied to apples on a turntable in a ventilated booth. The apples are supported on spikes through the calyx of the fruit prior to spraying. After the spray has dried, the stem of each apple is infested with five larvae. Ten to twenty apples are infested for each treatment. Infestation of treated apples is rotated so that only two larvae are placed on a treated apple before two more larvae are placed on an apple with a different treatment to insure randomization of test larvae used.

A thin ring of tangle foot is placed around the calyx to prevent the larvae from crawling down the spike or entering the calyx. The apples with infested larvae are placed in a holding room for 7 days after treatment, when they are scored for larval entries into the fruit and fruit stings. Water-treated checks are used in all tests. Guthion is used as the standard.

Adulticide Test:

Method is the same as described for the red-banded leaf roller. (See page 22).

*Estigmene acrea* - salt-marsh caterpillar

Tucson, Arizona

Method is the same as described for the cotton leaf perforator, (see page 23) except second- and third-instar larvae are used, and a 3:1 toxaphene-DDT mixture or Sevin is used as the standard.

*Heliothis virescens* - tobacco budworm

Brownsville, Texas

Spray Test:

Candidate insecticides are applied as emulsions or suspensions to potted cotton plants, as described for the cotton leafworm (see page 22). After the spray on the plants has dried, leaves are clipped from them and placed in covered petri dishes and five third-instar larvae are placed in each dish, with two dishes per treatment. Larval mortalities are recorded after 24, 48, and 72 hours. DDT is used as the standard.

*Heliothis zea* - bollworm or corn earworm

Brownsville, Texas

Spray Test:

Method is the same as described for the tobacco budworm. (See above).

Tucson, Arizona

Spray Test:

Emulsion sprays of the candidate insecticides are applied to potted cotton plants with a DeVilbiss hand-atomizer nozzle. Immediately after spraying, 10 to 20 second- to fourth-instar larvae are caged with each plant for a 72-hour period

and mortality counts are made after 24, 48, and 72 hours. DDT and Zectran are used as the standards.

Laphygma frugiperda - fall armyworm

Tifton, Georgia

Dip Test:

Oat leaves are dipped in solutions, emulsions, or suspensions of serial dilutions of the candidate insecticide. Twenty larvae, 3 days of age, are caged on 3 grams (12-15 leaves) of young oat plants which have been dipped in the serial dilutions and air-dried.

Each treatment is replicated twice. Tests are made with stainless steel bowl-type cages covered with polyethylene sheets. Mortality counts are taken at 24 and 48 hours. Results are based upon larval mortalities adjusted to the check and compared with a standard. Standards selected depend upon the type of compound being tested.

Fumigation Test:

The candidate insecticides (1/100 g.) in its purest form is weighed into a miniature dish placed on the bottom of a 1-gallon jar. The jar is closed with a standard screw-type lid in which a plastic screw thread (portion of a vial lid) has been cemented. Twenty-four hours later, a vial, containing twenty 3-day-old larvae feeding on oat leaves and restrained by a cheese-cloth cover over its open end, is screwed into the jar lid. Mortality records are taken after the larvae have been exposed to the insecticide for 24 hours. The test is repeated at further intervals of 1,2,4,8, and 16 days with lid openings capped between tests. The treatments are not replicated. Aldrin is used as a standard and in evaluating results. Adjustment of mortalities is based on controls.

Ostrinia nubilalis - European corn borer

Ankeny, Iowa

Artificial Diet Test:

Candidate insecticides are screened in the laboratory by confining 7-day-old European corn borer larvae on an artificial diet which has been treated with an insecticide suspension.

Plastic jelly cups (18 mm. deep X 26 mm. dia. bottom X 3 $\frac{1}{4}$  mm. dia. top) are used to confine the larvae during the test. The cups are closed with a paper-board cap lined with Saran. The Saran liner is necessary to keep the larvae from chewing through the cap.

Two milliliters of hot artificial diet are poured into each cup and allowed to cool to room temperature. After cooling, the cups containing the diet are stored in plastic bags at 40° F. until needed for tests.

The test insecticide suspensions are prepared by dissolving 100 mg of insecticide in 10 ml of acetone containing 10 mg Triton X-155 emulsifier. The emulsifiable concentrate is added to 90 ml of water to make a concentration of 100 ppm of actual insecticide. This suspension is diluted serially to make the more dilute test suspensions; 0.2 ml of test suspension is added to the cups and allowed to dry before introducing the larvae.

Five 7-day-old larvae, reared on artificial diet at 80°F and 85% r.h., are introduced into each cup. Each test is replicated four times. While under test, the larvae are held at 80°F and 85% r.h.

After 72 hours exposure in the treated cups, the larvae are counted and the percent mortality calculated.

Pectinophora gossypiella - pink bollworm

Brownsville, Texas

Contact-Spray Test:

Candidate insecticides are tested by exposing adult pink bollworms to spray deposits on cotton plants. Potted plants are placed on a turntable in a spray chamber and sprayed with a measured amount of an emulsion or suspension containing the technical material at a calculated application rate per acre. After the spray deposit has dried on treated plants, a laboratory cage is placed over each plant and 15 to 30 moths are released in each cage. Moth mortalities are recorded after 24, 48, and 72-hours. DDT or Sevin is used as the standard.

Residual Spray Test:

Method is the same as described in the contact-spray test above except treated plants are weathered in the open for elected time intervals (1 or more days) before they are returned to the laboratory and infested with adult pink bollworms. Mortalities are recorded after 24, 48, and 72 hours.

Prodenia eridania - southern armyworm

Brownsville, Texas

Spray Test:

Each side of a cotton leaf is sprayed in a Potter tower with 5 ml. of acetone solution of the candidate insecticide. After spraying, the leaf is cut in half and each half exposed in a petri dish to 10 fourth-instar larvae. Various concentrations up to 0.25 percent are used to give a range of kills. Each treatment is replicated 4 times. Moribund and dead larvae are recorded after 24 and 48 hours. DDT is used as the standard.

Spodoptera exigua - beet armyworm

Tucson, Arizona

Method is the same as described for the salt-marsh caterpillar. (See page 24).

Trichoplusia ni - cabbage looper

Brownsville, Texas

Spray Test:

Larvae about three-fourths grown are exposed on cotton plants treated in the same manner as described for the cotton leafworm (see page 22), with 10 to 20 larvae being used per plant. Larval mortalities are recorded after 24 and 48 hours and in some instances after 72 and 96 hours. Endrin is used as the standard.

Charleston, South Carolina

Dip Test:

Cabbage or collard leaves are dipped in solutions, emulsions, or suspensions of varying concentrations of the candidate insecticides. Third-instar larvae are then caged on sections of the treated leaves after the spray has dried. Each treatment is replicated 4 times in small plastic dishes containing one leaf section and 10 loopers. Results are based on adjusted larval mortality after 48 hours. Dibrom is used as the standard.

Tucson, Arizona

Method is the same as described for the salt-marsh caterpillar. (See page 24). Endrin is used for the standard.

Coleoptera

Anthonomus grandis - boll weevil

Brownsville, Texas

Contact-Spray Test:

Cages containing twenty 2- or 3-day-old square-reared adult boll weevils are placed in a horizontal wind tunnel and exposed to sprays of 5 ml. of acetone solution of the insecticide. Various concentrations up to 0.25 percent are used to give a range of kills. Each treatment is replicated 4 times. Moribund and dead weevils are recorded after 24 and 48 hours. Dieldrin, Guthion, and carbaryl are used as standards.

College Station, Texas

Systemic Test:

The technical candidate insecticide is dissolved in an organic solvent and mixed with agricultural-grade carbon so as to result in a 50-percent carbon dust (w/w). Cotton seeds are treated at the rate of 4 pounds of active ingredient per 100 pounds of seeds (equivalent to 1 pound per acre). A few drops of 4-percent methyl cellulose are added as a sticker to each group of 50 seeds. The seeds, methyl cellulose, and carbon-insecticide mixture are placed in a jar and mechanically rolled for 30 minutes. The seeds are then planted in 1-gallon cans of soil, 6 seeds per can. Upon emergence, the cotton plants are tested for insecticidal activity. Terminal leaves from the treated plants are removed and placed in 1/2-pint jelly dishes. Ten weevils are placed in each dish. Mortality of the weevils is observed for 3 days. These tests are continued on a weekly basis until the plants are no longer toxic to the weevils. All tests are replicated 2 to 3 times. Phorate at 4 pounds per 100 pounds of seed is used as a standard.

*Conoderus falli* - southern potato wireworm

Charleston, South Carolina

Soil Treatment Test:

Emulsions, suspensions, or solutions of the candidate insecticide are mixed with screened, air-dried soil in a ball mill. Twenty larvae are caged individually in salve cans, each containing the insecticide-soil mixture. As far as possible, equal numbers of larvae of different sizes are exposed to each dilution of the insecticide. Final results are based on adjusted larval mortality after 9 weeks. DDT and parathion are used as standards.

*Diabrotica balteata* - banded cucumber beetle

Charleston, South Carolina

Soil Treatment Test:

Emulsions, suspensions, or solutions of the candidate insecticide are mixed into screened soil with a ball mill. Second-instar larvae are caged(five per cage) in small plastic dishes containing the insecticide-soil mixtures. Each treatment is replicated in 5 cages. Results are based on adjusted larval mortality after 1 week. DDT and parathion are used as standards.

Hypera postica - alfalfa weevil

Beltsville, Maryland

Residue Test:

Serial dilutions of insecticide-acetone solutions (.1 ml/container) are applied to Number 32 Nalgene containers with lids. The containers are thoroughly shaken and set in a holding chamber with the lid off for 14 days. Ten adult weevils are placed in each container and held for 2 hours. The weevils are then taken out and placed in a plastic petri dish with food, and mortality counts taken at 24-hour intervals. All containers are discarded after each test. Ethyl Guthion and methoxychlor are used as standards.

Diptera

Aedes aegypti - yellow-fever mosquito

Gainesville, Florida

Systemic Test with Rabbits:

Materials which have shown systemic action in Livestock Insect Investigations are tested as oral systemics in rabbits against Aedes aegypti mosquitoes. Various dosages of the insecticide are administered to the rabbits by stomach tube and mosquitoes are fed on the treated rabbits.

Mosquitoes, 6 to 8 days old, which have not previously been given a blood meal, are immobilized in a cold room at approximately 32° F., usually the day before the tests. Lots of 20 females are placed in screen-wire cylinders, 2-3/4 inches in diameter and 8 inches in length. After the mosquitoes have recovered from chilling they are allowed to feed for 10 minutes, a sufficient time for engorgement, on treated rabbits on their backs in stanchions. One lot of mosquitoes is fed on each rabbit prior to treatment as a check. After treatment other lots are usually fed at hourly intervals up to 5 hours. If complete mortality is obtained in the lot fed 5 hours after treatment, lots are fed after 24 hours, 48 hours, and then weekly as long as there is high mortality. After feeding the mosquitoes are again immobilized in the cold room and those not completely engorged are discarded. Usually most of the mosquitoes engorge. Cylinders containing the engorged mosquitoes are then held in a box at about 80° F. for 24 hours. Wet turkish towels are laid over the box to increase the humidity. Mortality is determined 24 hours after feeding. Only engorged mosquitoes are considered in calculating percent mortality. The mosquitoes not capable of sustained flight are considered knocked down and moribund and are included with the dead.

Aedes sollicitans - salt-marsh mosquito

Gainesville, Florida

Contact Spray Test:

In tests with contact sprays, adult mosquitoes 1 to 3 days old are exposed to various concentrations of the insecticides in a wind tunnel. This apparatus consists essentially of a cylindrical tube 4 inches in diameter through which a column of air is moving at 4 m.p.h., drawn by a suction fan. The mosquitoes are confined in a cylindrical screen cage which is placed in the center of the tube. One-fourth milliliter of the insecticide in a kerosene solution is atomized into the mouth of the tube, and the mosquitoes are exposed momentarily as it is drawn through the cage. When necessary, acetone or other auxiliary solvents are used to assure complete solution of the compounds. The mosquitoes are then transferred to untreated screen holding cages and furnished with a honey-water solution. Mortality is recorded after 24 hours. All tests are run in duplicate, with 25 mosquitoes of mixed sexes in each cage.

Aedes taeniorhynchus - black salt-marsh mosquito

Gainesville, Florida

Contact Spray Test:

Method is the same as described for the salt-marsh mosquito. (See above).

Anastrepha ludens - Mexican fruit fly

Mexico City, Mexico

Residue Test:

Candidate insecticides are tested as emulsions, solutions, or suspensions by applying 2 ml. of the spray at varying concentrations to the bottom of a petri dish. After the spray has dried, the treated petri dish bottom is used as the top of a cage. The cage consists of an untreated petri-dish bottom and a cylindrical wall of 16-mesh screen. Three cages per concentration are used with 10 pairs of flies in each cage. Percent mortality is recorded 24 hours after the flies have been confined and LD-50 and LD-95 readings in micrograms per square centimeter of glass surface determined. Malathion is used as the standard.

Anopheles quadrimaculatus - common malaria mosquito

Gainesville, Florida

Larvicide Test:

Compounds are screened as mosquito larvicides by exposing early fourth-instar larvae to solutions or suspensions of the compound in water. The compounds are dissolved in acetone and added to water; water-soluble compounds remain in solution and the others become finely divided suspensions. Mosquito larvae are added to the treated water and mortality is determined after 24 hours of exposure. If 95- to 100-percent mortality occurs, at the initial concentration of 10 parts per million, additional tests are made to determine the minimum effective concentration. Under these conditions, the standard larvicide, DDT, is completely effective at a concentration of 0.01 p.p.m., but kills only 50-70 percent of the larvae at 0.005 p.p.m.

Classification of percent kill:

1. Less than 50 percent at 10 p.p.m.
2. 50-94 percent at 10 p.p.m.
3. 95-100 percent at 10 p.p.m.
4. 50 to 100 percent at 1 p.p.m.
- 4a. 95 to 100 percent at 0.01 p.p.m.

Residual Spray Test:

Acetone solutions of the insecticides are sprayed on 12" x 12" plywood panels at the rate of 100 mg. of active ingredient per square foot. The panels are tested 1 week after treatment, again after 4 weeks, and every 4 weeks thereafter until less than 70-percent kill is obtained.

Enough panels are sprayed with each insecticide to avoid the necessity of using any surface twice. In each test 20 female mosquitoes are exposed under half-sections of petri dishes on the treated panels for periods ranging from 5 to 120 minutes, after which they are transferred to cylindrical screen cages, provided with a honey-water solution in pads of absorbent cotton, and held for 24 hours, when mortality counts are made.

Ceratitis capitata - Mediterranean fruit fly

Honolulu, Hawaii

Topical Test:

Candidate insecticides are tested topically by making applications to the dorsal fruit fly thorax at a graded range of concentrations

to establish LD-50 and LD-95 levels. After topical treatments, the flies are held for 24 hours for observation and mortality counts. Most weight is given to the LD-50 value and the relationship between LD-50 and LD-95 levels. The dosage is calculated on the basis of the amount of toxicant per fly, not per weight, as is usually done. Mixed sexes are used since early tests indicated no significant difference between sexes. Weight is not used because this can be changed suddenly if the fly engorges on food or water before or regurgitates or defecates during the following treatment, or if the fly is a female and has been full of eggs and just deposited a large clutch. Malathion is used as the standard.

Residue Test:

A graded series of deposits of the candidate insecticide is applied to petri dishes. The deposits are allowed to dry overnight and then the dish is inverted over the top of wire mesh cages into which flies are introduced. Since these flies generally rest on the top of the cage, they are in contact with the insecticide until they drop. Mortality counts made after 24 hours of exposure provide information for establishing LD-50 and LD-95 levels. Again the LD-50 value is given the most weight, along with the relationship between it and the LD-95 level. Malathion is used as the standard.

Systemic Test:

Candidate insecticides are tested against fruit fly larvae in fruit by spraying young guava trees with fruit in all stages of development with 1 and 2 pounds of toxicant per 100 gallons to run-off point. Ripe fruits are picked at weekly intervals. The apical end is sliced off and 100 fruit fly eggs applied. The number of larvae recovered is compared with that recovered from untreated controls. By testing the fruit weekly, it is possible to determine whether a transfer of the toxicant to the fruit occurs, when it occurs, and how long it remains effective, along with the stage of development that the fruit was in at the time the spray was applied. Demeton is used as the standard.

In other tests, applications may be made to the soil or to the trunk to determine whether any translocation of toxicant takes place.

Fumigant Test:

Candidate fumigants are screened with laboratory-reared fruit flies at 1-ml. dosages in 5-gallon fumigatoriums. Following each treatment, fumigatoriums are aired for 1 hour before insects are removed. Fumigants giving 85% mortality or better are retested at three or more dosages to determine LD-50 and LD-95 values that are compared with values for ethylene dibromide.

Approximately 200 eggs are counted, placed on moist blotting paper in petri dishes, and held 16-20 hours at 80°F. before treatment. Mature larvae are also subjected to the fumigant by counting out 25 immediately before testing and sealing them in perforated, unwaxed paper cups. Unfumigated insects are used for controls. These are kept apart from the treated insects and held at 80°F. in a former vapor-heat room where conditions are practically stable. Mortality is determined after 48 hours by counting unhatched eggs or normally formed pupae in treated and untreated sets and corrected for mortality in the untreated sets.

Toxicant-Chemical Lure Test:

A 1-percent concentration of a soluble candidate insecticide is added to the most efficient chemical lure available for the male of the species. One drop of the lure plus the candidate toxicant is placed on a 0.25-cm. square piece of filter paper. The treated filter paper is fitted into a depression slide and covered with a 16-mesh monel screen. The procedure prevents contact by the fly with the treated lure except with its mouthparts as it reaches through the screen to feed on the lure or is contacted by toxic fumes. The slide is exposed in a quiet location where flies that are attracted will not be blown or frightened off and the time required for knockdown after feeding starts is used to evaluate effectiveness. Quick action is important to minimize amount of lure consumption. The same slides are re-exposed periodically to determine the duration of effectiveness. Dibrom or DDVP are used as the standards.

Cochliomyia hominivorax - Screw-worm

Mission, Texas

Beaker Test:

Candidate larvicides are tested against screw-worm larvae at three concentrations in duplicate beakers. Each beaker contains 15% citrated blood, 44.7% water, 0.3% formalin, and 40% fine ground lean meat. A quantity of a solution of the candidate larvicide in acetone/Tween 20 (9:1) is added to give a final concentration of 0.1 p.p.m., 1 p.p.m., or 10 p.p.m. The beakers are warmed for one hour at 90°F. and 85% R.H. and 25 or more newly hatched larvae are added to each. Results are recorded 24 and 48 hours later as immotile (all) or motile (few to all).

Cochlicomyia macellaria - secondary screw-worm

Kerrville, Texas

Systemic Test:

Candidate insecticides are screened as systemic insecticides by treating guinea pigs infested with 1-day-old larvae of the secondary screw-worm.

Candidate insecticides, formulated as 5-percent solutions in Tween-20, are administered orally and subcutaneously to two guinea pigs. If the guinea pig or the larvae are killed at the initial dosage (usually 100 mg/kg), lower dosages are tested until there is no insecticidal activity and/or no toxicity to the guinea pig. The standard, ronnel (Dow ET-57), is effective against secondary screw-worms as low as 50 mg/kg.

Culex tarsalis

Corvallis, Oregon

Contact Spray Test:

In tests with contact sprays, adult mosquitoes 2 to 4 days old are exposed to various concentrations of the insecticide in a wind tunnel. This apparatus consists essentially of a cylindrical tube through which a column of air is moving at 4 m.p.h., drawn by a suction fan. The mosquitoes are confined in a cylindrical screen cage which is placed in the center of the tube. One-tenth milliliter of the insecticide in a deodorized kerosene or kerosene-acetone (1:1) solution is atomized into the mouth of the tube, and the mosquitoes are exposed momentarily as it is drawn through the cage. They are then transferred to untreated screen holding cages and furnished with a sugar-water solution. Mortality is recorded after 24 hours at 80°F. and 60-65 percent relative humidity. All tests are run in duplicate, with 20 female mosquitoes in each cage.

Larvicide Test:

Compounds are screened as mosquito larvicides by exposing early fourth-instar larvae to solutions or suspensions of the compound in 250 ml. of distilled water. The compounds are dissolved in acetone and added to water; water-soluble compounds remain in solution and the others become finely divided suspensions. Mosquito larvae are added to the treated water and mortality is determined after 24 hours of exposure at 80°F. If 95-100 percent mortality occurs, at the initial concentration of 0.1 p.p.m., additional tests are made to determine the minimum effective concentration. Under these conditions, the standard larvicide, DDT, is completely effective at a concentration of 0.01 p.p.m., but kills only 50-70 percent of the larvae at 0.005 p.p.m. (In some tests parathion and/or malathion are used as standards).

Dacus cucurbitae - melon fly

Honolulu, Hawaii

Methods are the same as described for the Mediterranean fruit fly.  
(See page 31).

Dacus dorsalis - Oriental fruit fly

Honolulu, Hawaii

Methods are the same as described for the Mediterranean fruit fly.  
(See page 31 ).

Drosophila melanogaster

Beltsville, Maryland

Spray Test:

Medium-sized ripe tomatoes with one or two fresh slits, each slit about 1-1/2 inches long, are sprayed to the point of run-off with an atomizer-type sprayer. Eight or more tomatoes are sprayed with each candidate insecticide. The sprayed tomatoes are placed in 10x10x10-inch screen cages; two tomatoes to a cage are used for each replication. Unsprayed slit tomatoes are used for the check. A known number of drosophila adults are released in each cage. Records are taken on mortality after 24 and 48 hours.

Dust Test:

Slit ripe tomatoes are dusted in a tower which is approximately 3 feet tall and 10 inches in diameter. The dusts are released at the bottom of the tower at about 30 p.s.i. Eight or more tomatoes are treated with 250-500 mg. of each candidate dust. After about 5 minutes, the tomatoes are removed from the tower and placed in cages for the remainder of the test as described above for the spray test.

Oviposition Test:

Slit ripe tomatoes are dusted with the candidate insecticides in the same manner as described for dust test above. The tomatoes are removed from the dust tower and placed on paper plates; two tomatoes to a plate are used for each replicate. The plates with tomatoes are placed at random on a turn-table 5 feet in diameter. The dusted tomatoes are exposed for about 16 hours at 1 r.p.m. to several thousand drosophila adults that have been released in the room where the turntable is located. At the end of the exposure, the tomatoes are examined under a binocular microscope and the number of eggs laid in each slit is recorded.

Tifton, Georgia

Fumigation Test:

Method is the same as described for the fall armyworm. (See Page 25).

Gasterophilus spp. = bot flies

Kerrville, Texas

Animal Systemic Test:

In initial tests, horses are treated orally with candidate insecticides administered with a stomach tube. For a week after treatment feces from the individual horses are collected and bots recovered. At the end of the week, horses are sacrificed and the gastrointestinal tracts examined for bots. Control is calculated by comparing numbers of bots expelled with numbers remaining in the horses. Dipterex at 40 mg/kg for 1 day in feed is very effective against bots and is used as the standard.

Haematobia irritans = horn fly

Kerrville, Texas

Laboratory evaluation techniques are currently under study. Present evaluations are made by herd spraying. Data includes pre- and post-treatment average counts of flies on 15 animals per herd. A candidate is considered to have failed when there are 15 flies per head on the post-treatment count.

Hypoderma bovis = northern cattle grub

Kerrville, Texas

Animal Systemic Test:

Compounds that show systemic activity in guinea pig screening tests are further evaluated in cattle. A compound is administered to a few grub-infested cattle at the highest dosage considered non-lethal to cattle. In initial tests, the compound is given orally (in capsule or drench); subsequent treatments may be intramuscular or dermal. After treatment, the animals are maintained on pasture and examined each month for encysting grubs. Cumulative counts of grubs are obtained by recording the location of each grub on a map outlining the back of each animal. Certain animals in each group are not treated; percent control is calculated by comparing grub counts in these animals with those of the treated animals. The treatments are made when the grubs are deep in the tissues, after the heel fly season and before grubs appear in the animals' backs. Two groups of cattle are available for these tests. One group of calves and yearlings comes from pastures at Camp Stanley, Texas, where the heel fly season lasts from January to March, and grubs (Hypoderma lineatum) appear in the animals' backs in September. The other group consists of calves imported from the northern states, where the heel fly cycle

is 2 months later, and grubs (H. lineatum and bovis) begin to appear in their backs in December-January. Treatments of the Texas cattle are usually made in July; those of the imported cattle are usually made in November.

Toxicity studies by cooperating veterinarians of the Animal Disease and Parasite Research Division furnish data on the highest dosage that is non-lethal to cattle; the veterinarians also observe cattle in systemic tests for evidences of toxicity during test.

The standard, ronnel (Dow ET-57), controls 85-95 percent of the grubs when administered orally at 110 mg./kg.

Hypoderma lineatum - common cattle grub

Kerrville Texas

Animal Systemic Test:

Method is the same as described for the northern cattle grub. (See page 36).

Liriomyza sp. nr. commeliniae - a leaf miner

Charleston, South Carolina

Dip Test:

Solutions, emulsions, or suspensions of the candidate insecticide are tested at varying concentrations. Twelve cowpea seedlings (3 per pot) infested with leaf miner larvae are dipped in each concentration. Results are based on the percent reduction in numbers of pupae obtained from the seedlings. Parathion is used as the standard.

Musca autumnalis - face fly

Beltsville, Maryland

Residue Test:

Five milliliters of an acetone solution containing 0.56 mg./ml. of the candidate insecticide are applied to the inner surface of a pint glass fruit jar. Jars are treated in duplicate, rolling the solution evenly over the bottom and sides until the acetone dries, leaving a deposit of 10 mg./sq. ft. A glass plate used as a top for the jar is also given the same coating. Twenty or more flies (mixed sexes) are then confined

in the jars for five minutes after which they are removed to cages and held for 24 hours at which time mortality of both sexes is determined. Any candidate giving a kill of approximately 50 percent or more is retested at lower dosages. DDT is used as the standard.

Musca domestica = house fly

Beltsville, Maryland

Residue Test:

Method is the same as described for the face fly (see above).

Gainesville, Florida

Contact-Spray Test:

In tests with contact sprays, female house flies 4 to 5 days old are exposed to various concentrations of the insecticides in a wind tunnel. This apparatus consists essentially of a cylindrical tube 4 inches in diameter through which a column of air is moving at 4 m.p.h., drawn by a suction fan. The flies are confined in a cylindrical screen cage which is placed in the center of the tube. One-fourth milliliter of the insecticide in a kerosene solution is atomized into the mouth of the tube, and the flies are exposed momentarily as it is drawn through the cage. When necessary, acetone or other auxiliary solvents are used to assure complete solution of the compounds. The flies are then transferred to untreated screen holding cages and furnished with a honey-water solution. Knockdown is recorded after 10, 30, and 60 minutes and mortality after 24 hours. All tests are run in duplicate, with 20 female house flies in each cage.

Bait Toxicant Test:

Tests as bait toxicants are conducted by mechanically mixing dry granulated sugar with an acetone solution of the experimental compound, allowing the acetone to evaporate, and exposing 5 grams of the mixture to 20 female house flies in a screen cage. The toxicants are tested at concentrations of 1.0, 0.1, and 0.01 percent. Mortality is recorded at 1, 2, 4, and 24 hours.

Residual Spray Test:

Acetone solutions of the insecticides are sprayed on plywood panels at the rate of 100 mg. of active ingredient per square foot. The panels are tested 1 week after treatment, again after 4 weeks, and every 4 weeks thereafter until less than 70% kill is obtained. Enough panels

are sprayed with each insecticide to avoid the necessity of using any surface twice. In each test 20 female house flies are exposed under half-sections of petri dishes on the treated panels for periods ranging from 5 to 120 minutes, after which they are transferred to cylindrical screen cages, provided with a honey-water solution in pads of absorbent cotton, and held for 24 hours, when mortality counts are made.

Oestrus ovis - sheep bot fly

Kerrville, Texas

Animal Systemic and Drench Test:

Sheep are treated orally with candidate insecticides in capsules or by drenches. Four to 14 days after treatment the sheep are sacrificed and the nasal chambers examined for live and dead grubs. Untreated sheep of similar age and condition from the same herd are also sacrificed and the number of grubs recorded. Control is calculated by comparing numbers of live bots in the treated and untreated sheep.

Phormia regina - black blow fly

Kerrville, Texas

Systemic Test:

Method is the same as described for the secondary screw-worm.  
(See page 33).

Stomoxys calcitrans - stable fly

Kerrville, Texas

Animal-Protectant Spot Test:

Candidate insecticides are screened as animal protectant sprays by the spot test method. Areas 6 inches in diameter on the side of a cow are sprayed with 5 ml. of a 0.5- and a 5.0-percent acetone solution of the compound. Materials not soluble in acetone are applied in other solvents. During the winter months the hair on each area is clipped so that the flies can reach the skin to feed, but clipping is not necessary when the animals are in summer coat. Five or six test areas, spaced on either side of an animal, are positioned so that cross-contamination is unlikely. The animals are confined in individual temperature-and humidity-controlled stalls and subjected to sunlamp exposure 4 hours daily through the test period. Cages, made by soldering screen

wire in a mason-jar ring are used to confine adult stable flies to the treated spots. Twenty-five 3- to 6-day-old female flies that have not fed for about 18 hours are exposed to each spot for 20 minutes. After exposure the flies are moved to a constant-temperature room for holding at 72° F. and 65-percent relative humidity. Toxicity is measured by the percent mortality of the flies 24 hours after exposure. When less than 90% of the flies are dead, the compound is considered to have failed as a toxicant. Methoxychlor at 0.5 percent, which is effective for 8 days, is used as the standard.

Compounds are rated according to the following classification:

Class I	Ineffective at 1 day
Class II	Effective at 1 day
Class III	Effective for 2-7 days
Class IV	Effective for 8 or more days

Systemic Test:

Candidate insecticides, formulated as 5-percent solutions in Tween-20, are administered orally and subcutaneously to two guinea pigs. Stable flies are fed on the guinea pigs 4 and 24 hours after treatment. Engorged stable flies are held for 24 hours to see whether they are affected by the blood they ingested. If the guinea pigs or stable flies are killed at the initial dosage (usually 100 mg./kg.), lower dosages are tested until there is no insecticidal activity and/or no toxicity to the guinea pigs. The standard, ronnel (Dow ET-57), is effective against stable flies at as low a dosage as 50 mg./kg.

Siphonaptera

Xenopsylla cheopis - oriental rat flea

Gainesville, Florida

Residue Test:

Strips of Whatman No. 1 or 2 filter paper, 15 mm. by 50 mm., (one end tapered), are impregnated with 0.13 ml. of an acetone solution containing 0.031 percent of an insecticide. This impregnation rate produces an equivalent of 5 mg. of toxicant per square foot. After drying for 1 hour, each treated paper (tapered end down) is inserted in and to the bottom of a glass test tube (18 x 150 mm.). Ten adult fleas of the normal colony, newly emerged and unfed, are placed in each test tube, which is then capped with organdy cloth. Mortality is recorded after 24 hours of exposure. After the exposure period, the test fleas are removed and treated papers are aged in the tubes and tested at various time intervals.

Dust Test:

Thirty grams of a standard pulverized soil of high organic content is placed in the top half of a 100-mm. petri dish and leveled. The surface of the soil is dusted in a dust tower with a pyrophyllite powder at the rate of 20 grams per square meter (2 gm./sq.ft.). Powders containing 0.1, 1.0, and 5.0 percent of the insecticide are used. The exposure chamber consists of a plastic dish, 95 mm. in diameter and 70 mm. high, with a circular opening 25 mm. in diameter cut in the bottom. The chamber is inverted and inserted into the treated soil, so that the soil becomes the floor of the exposure chamber and the opening is at the top. Fifty newly-emerged adult fleas are introduced through the opening in the top of the chamber and exposed on the treated soil for a period of 24 hours, at which time knockdown and mortality readings are recorded. Malathion is used as the standard insecticide and untreated pyrophyllite and undusted soil are used as checks.

Secondary evaluations may be conducted with the most promising insecticides at additional concentrations.

Systemic Test with Guinea Pigs:

Materials which have shown systemic action in Livestock Insect Investigations are tested as oral systemics in guinea pigs against oriental rat fleas, Xenopsylla cheopis. Guinea pigs are treated by stomach tube with various dosages of the insecticide and fleas are fed on the treated guinea pig.

Two-day old unfed fleas are transferred with aspirator from rearing jars to vials 1 inch in diameter by 3-3/4 inches in depth. After one lot of 30 fleas has been counted into each vial the vials are covered with 64-mesh organdy cloth which has been washed to remove the sizing. This cloth retains the fleas but allows them to feed through it. A dark cloth covers each vial, enabling the fleas to feed in darkness. The fleas are allowed to feed for 10 minutes, usually a sufficient time for engorgement, on the clipped belly while the guinea pig is held on its back in a stanchion.

After a 10-minute feeding period the fleas in the vials are anesthetized with carbon dioxide and examined under a microscope to determine the number that have fed. If more than 20 have fed, 20 well-fed fleas are returned to the vial and the rest are discarded.

If less than 20 feed, the fleas are returned to the vial, placed on the guinea pig for an additional 10 minutes and examined again. The unfed fleas are discarded and the fed fleas, up to 20 in numbers, are returned to the vials. The vials are held at a temperature of 80° F. and a relative humidity of 70%. Mortality is determined 24 hours after feeding. The knocked-down fleas are counted with the dead in determining percent mortality. If more than 90% mortality is obtained in the lot fed 5 hours after treatment, lots are fed after 24 hours, 48 hours, and then weekly as long as there is high mortality.

#### Test Methods - Attractants

##### Orthoptera

###### Blattella germanica - German cockroach

Gainesville, Florida

Cockroach traps are made by applying a mixture of liquid and white petrolatum to glass crystallizing dishes (125 mm. dia. x 65 mm. height) and placing sleeves of corrugated cardboard around the outside walls. Two traps, one containing 2 grams of the candidate attractant and another with 2 grams of the standard (Coca Cola syrup) are placed in a 15-1/2 gallon rectangular tub (galvanized metal) fitted with a metal ring sealed to the top with adhesive or masking tape. The ring is 2 inches wide and the outside dimension is the same as that of the tub. When in place, the ring makes a flange projecting 2 inches toward the center of the tub. The under surface of this flange is greased with a mixture of equal parts of liquid and white petrolatum-- a procedure which effectually prevents the escape of cockroaches. Each tub is charged with 200 adult cockroaches; food, water, and harborages are available. The tub is covered with a black cloth and after an exposure period of 1 hour, the cockroaches in each trap are counted. Each test consists of 4 replications.

Diptera

Anastrepha ludens - Mexican fruit fly

Mexico City, Mexico

Spot Test.

Candidate attractants are divided in two groups: coded compounds from the Synthesis Investigations, Pesticide Chemicals Research Branch, and fermentation products from the Utilization Research and Development Division.

Compounds from Synthesis Investigations are exposed undiluted in a screened olfactometer 9' x 9' x 9', enclosed in a room of 2145 cubic feet. Lighting is indirect with white, daylight, and yellow fluorescent lamps arranged alternately. Intensity in center of olfactometer is one foot-candle. Fly population is maintained at approximately 60,000 to 80,000 about equally divided as to sex. Small blotting paper discs, 1" diameter, are stapled to cellophane films 8" x 10". The films are hung on the exterior hooks of the olfactometer wheel for a period of 20 minutes and compared with the standard fermenting lure placed in the same manner. Counts are made at 10 and 20 minutes. An additional exposure of the same compounds aged 1 day are made. Cellophane films with the chemical in the blotting paper are maintained during the aging period at room temperature. Classifications are based on number of flies attracted in comparison with the standard and are the following: None (N), Less than (L), Equal to (E), More than (M) and Heavy (H).

Fermentation products are exposed on blotting paper discs of 1" diameter fastened with Scotch tape to 3" x 3" squares of transparent plastic. The squares are hung on a wire around the periphery of the olfactometer 3" from the screen sides and 5' above the floor. Exposure is for 30 minutes. Count of flies resting on the blotting paper discs are made at intervals of 15 and 30 minutes and test compounds are rated against the standard fermenting lure and protein lure included in each trial. Tests are made 0, 1, 3, and 6 days after receipt of the compounds. During the aging period the materials and controls are kept at 26° C.

Aqueous Solution Test

Tests are conducted in the olfactometer with compounds dissolved in water or ethanol and emulsified in water to give a concentration of 0.1 percent or 1-percent of the test compound. Three replicates are exposed in small invaginated glass traps (McPhail traps) of 50 cc. capacity for one hour at 25° C. and 50-percent relative humidity, and are compared with water and with the standard fermenting lure included in the cage.

#### Field Wick Test

Wicks with five drops of a 100-percent concentration of the liquid materials or 0.25 grams in 1 milliliter of water or ethanol of the solid materials are suspended over water in invaginated glass traps (McPhail traps) of 300 cc. capacity and exposed in mango trees from five to seven days. The standard fermenting lure is used as a control.

#### Field Solution Test

Fermentation products are dissolved in water to give a concentration of 1-percent of the test compound and at the same concentration in water plus 8-percent light brown sugar in invaginated glass traps for periods of five to seven days in mango trees. The standard fermenting lure is used as a control.

#### Ceratitis capitata - Mediterranean fruit fly

Honolulu, Hawaii

#### Liquid Test:

Candidate lures are emulsified in water and tested at 0.1 percent concentration on a revolving trap suspension in an outdoor cubicle cage containing thousands of fruit flies. Usually all three of the Hawaiian species, the Mediterranean fruit fly, the oriental fruit fly, and the melon fly are used. The usual exposure period for a test is 1 hour. Traps containing water are included as a check and each treatment is replicated 3 times. Lures are rated Class 3 if the catch exceeds 50 times that of water alone, Class 2 for males if between 10 and 50, for females if between 6 and 50, and Class 1 if less than 10 for males and less than 6 for females. Lures that catch 50 or more times the number caught by water are further evaluated by placing 0.5 ml. of the lure on one end of a 1-1/2 inch section of a cotton dental roll taped to a rectangular sheet of kraft paper which is kept revolving on a hexagonal wheel inside the olfactometer cage for 15 to 20 minutes. In each test, efficiencies are calculated against catches made by trimedlure, methyl eugenol, or cue-lure, for the Mediterranean fruit fly, the oriental fruit fly, and the melon fly, respectively. The various standard lures are each given a value of 100.

Ratings are approximate since the flies are not killed or collected from the wicks and their numbers may be more than can be easily counted or estimated. Methyl eugenol is used in the cage only to check against a lure that shows very strong attraction to the oriental fruit fly since it is so highly attractive to that species and will invariably attract more than 1,000 flies long before the end of the usual 15-minute exposure period. When a solid lure is applied to the wick, it is usually diluted in alcohol or acetone, and the solvent is allowed to evaporate before the test is run.

Duration of attractiveness on the wicks is tested by placing the papers containing the wicks in a well-ventilated room after the initial test run. The wicks are then re-exposed at intervals over a period of time until they no longer show effectiveness. Information thus obtained has been found to agree very well with results subsequently obtained in traps tested in the field, and so greatly reduces the amount of field testing required.

#### Bait Spray Attractant Test

Bait sprays are effective after the deposits have dried on foliage. There is very little correlation between the attractiveness of the dried deposits and that of the same attractant in the liquid form. Therefore, the effectiveness of attractants in liquid baits cannot be used to estimate their effectiveness as bait-spray. All candidate bait-spray attractants are exposed in combination with malathion by applying a measured quantity of the concentrated mixture in droplets on the top and bottom surfaces of a cluster of guava leaves growing from a guava twig tied across the top of a 3' x 3' tray. The tray contains a coarse-mesh screen bottom over a fine-mesh screen so that the flies as they die fall through the top screen and escape predation by birds. The legs supporting the tray 2 to 3 feet above ground along with the trunk of the trees are treated with tanglefoot to prevent ants from carrying off the flies. By replicating the treatments in young guava orchards, reliable results are generally obtained with 6 to 10 replicates in tests of two weeks' duration provided rains do not remove deposits. The method of testing gives information on the duration of the attraction under different weather conditions.

If fly populations are too low to give adequate data, the treatments are reapplied and the test extended for one or more additional two-week periods. Most tests were run against mixed populations of the melon fly and the oriental fruit fly. There are no significant medfly populations in the guava areas available near Honolulu. When information is needed on this species, releases are made in the orchards while the tests are in operation. Malathion alone was originally used as a check but since it caused no drop of flies on the trays by itself it is used in combination with the standard formula of Staley's Insecticide Bait No. 7, representing the acid hydrolyzates, or Type M Fleischmann's yeast hydrolyzate, representing the enzymatic types of protein hydrolyzate. Candidate bait-spray attractants are always compared on the basis of equal solids content; however, the advantage or disadvantage of increasing or decreasing the amount is also tested by this method.

Insecticides as bait spray toxicants cannot be reliably tested by this method because of differences in the time required to knock down and kill flies. However, since the application of insecticides by themselves rarely drop a significant number of flies on the trays compared to the hundreds of thousands that collect there and die when baits were added, it is possible to partially evaluate toxicants by considering any toxicant worthy of subsequent large-scale field testing that caused a substantial kill of flies on the trays.

Toxicants that when combined with bait sprays caused a good kill for several days and then stopped giving increased kills of flies before the standard treatment, are suspected of losing toxicity more rapidly than the attractant was lost and were thus considered dangerous to use in bait spray formulas (for example, malathion, as recommended, always outlasts the protein hydrolyzate bait-spray attractant. Dipterex does not.)

Dacus cucurbitae - melon fly

Honolulu, Hawaii

Methods are the same as described for the Mediterranean fruit fly.

Dacus dorsalis - oriental fruit fly

Methods are the same as described for the Mediterranean fruit fly.

Drosophila melanogaster

Beltsville, Maryland

The candidate materials are screened in pint jar traps on a turntable 5 feet in diameter in a room measuring 15 x 11-1/2 x 9 feet where several thousand drosophila adults have been released. Each trap is baited with a 1-inch section of dental roll impregnated with 15 - 30 drops of material; each material is tested in duplicate traps. The traps are placed at random on the table and run at about 1 r.p.m. for 1-1/2 hours. At the end of the test a record is made on the number of flies captured in trap. The standard is a fresh bait of 10% sugar, 4% active dry yeast, 1% apple cider vinegar and water.

Test Methods - Repellents

Acarina

Amblyomma americanum - lone star tick

Gainesville, Florida

Patch Test:

Cotton twill patches, 4 inches square, are impregnated with 0.222 grams (2.0 g./sq.ft.) of the material to be tested. The patch is attached to a wire paddle and held in a vertical position in

a pen infested with nymphal lone star ticks. The number of ticks crossing the bottom 2 inches of the treated cloth and a comparable untreated cloth in a 1-minute exposure period is used to compute the percent repellency. In this procedure, tests are repeated at 1-week intervals to determine the number of days a treated patch shows a minimum of 80-percent repellency.

Orthoptera

Blattella germanica - German cockroach

Gainesville, Florida

Five milliliters of a 2% solution of the candidate material in acetone is applied to the inside of a one-half pint cylindrical paperboard carton. A hole 3/4 inch in diameter is cut in the carton to give the cockroaches access to food and water, which are placed inside. The carton is set in an enamel dishpan containing 10 male and 10 female German cockroaches. A mixture of liquid and white petrolatum is applied as a 2-inch band along the top margin of the pan to prevent the cockroaches from escaping. A count of the number of cockroaches in the treated carton is made after intervals of 1, 3, and 7 days, and weekly thereafter, until the compound is found to be ineffective. Effectiveness is based on the length of time before more than 50% of the cockroaches consistently enter the treated carton. Dead cockroaches are replaced 24 hours prior to each day's count. Fencholic acid (ENT-14249) is tested as a standard concurrently with the candidate repellents, and acetone-treated cartons are tested as checks.

Beltsville, Maryland

The candidate repellent is tested at 1 percent in acetone. One milliliter of the solution is pipetted into a cardboard box two inches square and one inch deep, and the sides of which have V-shaped openings cut in the open ends so that the cockroaches may enter and rest on the inside as well as the outside surfaces of the box. The treated box and an untreated box are inverted and placed in a crystallizing dish in which 10 male and 10 female German cockroaches are confined. Counts are made daily of the number of insects in each box. The insects are shaken out and the position of the boxes reversed after each count. Six daily counts are made during a week's time. The percent of insects in the treated box of the total number in both boxes is calculated. Zero indicates complete repellency whereas fifty percent or more indicates no repellency. Fencholic acid is tested as a standard concurrently with the candidate repellent.

Hymenoptera

Apis mellifera - honey bee

Tucson, Arizona

A metal can-like container approximately 5 1/2" in diameter and 7" long is used as a vaporizing unit. The telescoping lid of the container and clothespin clamps attached to the outside of the container hold a narrow strip of paper toweling treated with a small quantity of the candidate repellent. An air stream from a compressor regulated to less than 1 lb. pressure is piped into the container so it is directed against the repellent on the paper. The fumes of the compound are then conducted to a transparent funnel made of plexiglas which is held over the bees on a comb. When the vapors strike the bees they react immediately according to the repellency of the compound. The reaction is compared with that produced by propionic acid and evaluated accordingly. At intervals, tests are repeated on each compound several times to evaluate the uniformity of the response.

Promising repellents found by the above method are further tested with foraging bees and finally in field trials on alfalfa plots, or in other applications.

Diptera

Aedes aegypti - yellow-fever mosquito

Gainesville, Florida

Treated stocking test:

Compounds are screened as mosquito repellents by exposing arms covered with treated cotton stockings in cages of Aedes aegypti mosquitoes. The stockings are treated with a 10-percent solution of the compound in a volatile solvent, usually acetone, at 3.3 grams of the compound per square foot. Two hours after treatment, the stockings are exposed for 1 minute on the arm of a human subject in a cage of mosquitoes. If less than 5 mosquitoes bite the subject through the stocking, the test is repeated after 24 hours, and then at weekly intervals until 5 bites are received in 1 minute. Under these conditions the standard repellent, dimethyl phthalate, is effective for 11 to 22 days, and an exceptionally good repellent, 2-butyl-2-ethyl-1, 3-propanediol, was effective for 196 days.

Classification of effectiveness:

1. Ineffective on the initial test (5 or more bites in 1 minute).
2. Effective for 1 to 5 days.
3. Effective for 6 to 10 days.
4. Effective for more than 10 days.
- 4a. Effective for more than 21 days.

Musca autumnalis - face fly

Beltsville, Maryland

Candidate repellents are evaluated in the laboratory by a modification of the sandwich bait method. Two thin strips of moist cow manure (a natural attractant of face flies) are spread on a piece of cardboard 2" x 3". One strip is covered with a 1-inch strip of lens paper which has been soaked in acetone and the other is covered with a strip soaked in 1-percent acetone solution of the candidate material. The "sandwiches" thus prepared are held in aluminum folders and placed near the front of a cage containing face flies. Counts of the number of flies on the untreated and treated surface are made at 1-minute intervals for 10 minutes. The percentage of flies on the treated surface of the total number of flies attracted to both strips is calculated. Zero percent indicates a superior repellent. Fifty percent or over indicates no repellency. The tests are replicated at least twice.

Stomoxys calcitrans - stable fly

Kerrville, Texas

Animal Protectant Spot Test:

Candidate repellents are screened as animal protectant sprays by the spot test method. An area 6 inches in diameter on the side of a cow is sprayed with 5 ml. of an acetone solution of the compound. Materials not soluble in acetone are applied in other solvents. During the winter months the hair on each area is clipped so that the flies can reach the skin to feed, but clipping is not necessary when the animals are in summer coat. Five or six test areas are spaced on either side of an animal, and are positioned so that cross-contamination is unlikely. The animals are confined in individual temperature and humidity controlled stalls and subjected to sunlamp exposure 4 hours daily through the test period.

Cages, made by soldering screen wire in a mason-jar ring are used to confine adult stable flies to the treated spots. Twenty-five 3- to 5-day-old female flies that have not fed for about 18 hours are exposed to each spot for 20 minutes. After exposure the flies are moved to a constant-temperature room for holding at 72° F. and 75-percent relative humidity.

Repellency is measured by subtracting the percentage of flies that feed from 100 percent. When more than 20 percent of the flies feed, the compound is considered to have failed as a repellent. Compounds are tested for repellency at a 5.0-percent concentration, and 0.05-percent pyrethrins, which is effective

for 4 days, is used as a standard of comparison. Compounds are rated according to the following classification:

Repellency (based on results at 5.0 percent)

Class I	Ineffective at 1 day
Class II	Effective at 1 day
Class III	Effective for 2-3 days
Class IV	Effective for 4 or more days

Test Methods - Chemosterilants

Acarina

Tetranychus telarius - two-spotted spider mite

Brownsville, Texas

Lima bean seedlings infested with two-spotted spider mites are exposed on a turntable in a wind tunnel to sprays of 5 ml. of a solution containing 2 gm. test material, 10 ml. of ethyl alcohol, and 88 ml. of solvent; dilutions are made from the stock solution if required. Treated plants are held for 24 hours for the mites to feed and move about before adult females are removed and placed on untreated lima bean leaf disks 2 cm in diameter; white filter paper 3 cm in diameter are placed between the bean leaf disks and wet cellulose sponges. The females are allowed to remain on the disks for 48 hours and then are destroyed. Eggs are covered and held for hatching. Ten adult females are used per disk; each treatment is replicated 3 times. Apholate is used as the standard.

Diptera

Anastrepha ludens - Mexican fruit fly

Mexico City, Mexico

Test flies are maintained in 8 x 8 x 8 in. cage. Candidate chemosterilants are dissolved in acetone and added to food medium consisting of 4 parts of granulated sugar to 1 part orange juice crystals neutralized with NaOH prior to addition of the test material. The mixture is homogenized in a mortar and the acetone is allowed to evaporate. Feeding begins with fly emergence and is continued for a 20-day period. Flies are egged at 13 and 20 days of age and mortality recorded at weekly intervals. Compounds are evaluated on the basis of total mortality, egg production, and hatch response.

Cochliomyia hominivorax - screw-worm

Mission, Texas

Candidate chemosterilants are tested against screw-worm flies by the oral treatment of adults less than 24 hours old. Flies are fed a freshly prepared quantity of sugar syrup containing 1% of the candidate chemosterilant daily for 5 days. On the eighth day following oral treatment, females are given the opportunity to lay eggs which are subsequently observed for hatching. The criterion for further study of a compound is no oviposition or the failure of eggs to hatch.

Musca domestica - house fly

Gainesville, Florida

Adult Test:

Adult flies are given the candidate chemosterilants in granulated sugar and/or regular fly food (consisting of 6 parts of sugar, 6 parts of powdered nonfat dry milk, and 1 part of powdered egg yolk). Fly food or sugar containing 1% of the chemosterilant is prepared by adding 6 ml. of a solution or suspension of the chemical in a volatile solvent to 10 grams of the food. The solvent is allowed to evaporate for 4 to 6 hours, and the dried sugar or fly food is repulverized. The treated diet, with a container of water, is placed in cages containing 100 newly-emerged adult flies. Cages of 100 flies, containing untreated fly food or sugar, are used as checks. After 3 days the flies are examined to note any mortality caused by the chemosterilant, and untreated regular fly food is added to those cages of flies containing the treated sugar diet to provide protein for egg development. When the flies are 6 to 7 days old, 1/2 inch of moist CSMA medium in a soufflé cup is placed in the cage for oviposition. Four to six hours later, the soufflé cup with the oviposition medium is filled with water and stirred to break up the egg masses. A random sample of 100 eggs is collected and placed on a small piece of wet black cloth, which is laid on the top of moist larval medium in a rearing container. If no eggs are laid, oviposition medium is offered again at intervals of 1 or 2 days until it has been offered five times or the flies have oviposited. After the eggs are exposed on the larval medium for 2 or 3 days, the percent hatch is determined. The larvae that hatch crawl from the cloth into the rearing medium, and about a week after oviposition the pupae are counted to determine the number of larvae that have reached the pupal stage of development.

MARKET QUALITY RESEARCH DIVISION

Test Methods - Insecticides

Lepidoptera

Plodia interpunctella - Indian-meal moth

Savannah, Georgia

Direct Contact Test:

Indian-meal moth larvae are treated by topical application. Larvae are anesthetized by placing them in a Büchner funnel and introducing carbon dioxide through a tube from a pressurized tank. The larvae are picked up individually by means of a suction tube and 0.5 microliter of the insecticide formulation applied to the dorsal thoracic region by means of an ISCO microapplicator with 1/4-cc. syringe and 27-gauge needle. The larvae are then placed in 1/2-pint jars, each containing a 1-inch piece of filter paper saturated with 30-percent glucose solution. The jars are held in room maintained at a temperature of 80° F.  $\pm$  2° and a relative humidity of 60 percent  $\pm$  5 for the post-exposure observations.

Coleoptera

Attagenus piceus - black carpet beetle

Savannah, Georgia

Direct Contact Test:

The test compounds are formulated in a 50-50 mixture (w/w) of Deobase and tetrachloroethylene. Malathion is used as the standard for comparison. The insects are placed in open petri dishes. Four dishes containing 10 insects each are placed on the paper-covered floor of each of four stainless steel settling towers (6 ft. high and 2 ft. in diameter) and the doors and exhaust checks are closed. The insecticide formulation is introduced through an aperture in the center of the top of each tower. The spray is allowed to settle for 1/2 hour. The treatment is conducted at a temperature of 75° to 80° F. As a check, insects are exposed to the solvent alone. The insects are removed from the towers, transferred to clean petri dishes, and maintained at a temperature of 80° F.  $\pm$  2° and a relative humidity of 60 percent  $\pm$  5 for post-exposure observations.

Residue Test:

Strips of aluminum foil laminated to 40-pound kraft paper, 3 by 12 inches, are treated with acetone solutions of the candidate insecticide by means of a Gardner automatic blade applicator. The paper surface of four strips each and the aluminum surface of four strips each are treated at several rates of application. Malathion is used as the standard. Twenty-eight days after the surfaces are treated, four open-end glass cylinders, 2.5 inches in diameter and 3 inches in height, are placed on each treated surface. Ten black carpet beetle larvae are introduced into each cylinder, exposed to the treated surface for 24 hours, and then transferred to clean petri dishes for post-exposure observations. Knockdown is recorded 24 hours after exposure. Knockdown and dead-plus moribund are recorded 168 hours after exposure.

Vapor Test:

Acetone solutions of the candidate insecticide are applied to the inner surface of 1-quart Mason jars. Jars are treated in quadruplicate with each insecticide at a rate of 200 mg/sq. ft. by rolling the solution evenly over the bottom and sides (up to the neck) until the solution will no longer run. The treated jars are stored open for 4 hours under a hood to allow any remaining acetone to volatilize. Ten black carpet beetle larvae are placed in crystallizing dishes, each covered with a canopy. The dishes are placed on lids and the jars are inverted over the dishes. This allows the insects to be exposed to the atmosphere within the jar without coming in contact with the treated surface. Knockdown is recorded at 24 hours' exposure. Knockdown and dead-plus-moribund are recorded at 360 hours' exposure. Lindane and malathion are used as standards.

Direct Contact Test (Topical Application):

Larvae are anesthetized by placing them in a Büchner funnel and introducing carbon dioxide through a tube from a pressurized tank. The larvae are picked up individually by means of a suction tube and 0.5 microliter of the insecticide formulation is applied to the dorsal thoracic region by means of an ISCO microapplicator with 1/4-cc. syringe and 27-gauge needle.

As a check, insects are treated in a similar manner using solvent alone. The insects are placed in clean petri dishes and maintained at a temperature of 80° F.  $\pm$  2° and a relative humidity of 60 percent  $\pm$  5 for postexposure observations.

Tribolium confusum - confused flour beetle

Savannah, Georgia

Direct Contact Test:

The test compounds are formulated in a 50-50 mixutre (w/w) of Deobase and tetrachloroethylene. Malathion is used as the standard for comparison. The insects are placed in open petri dishes. Four dishes containing 10 insects each are placed on the paper-covered floor of each of four stainless steel settling towers (6 ft. high and 2 ft. in diameter) and the doors and exhaust checks are closed. The insecticide formulation is introduced through an aperture in the center of the top of each tower.

The spray is allowed to settle for 1/2 hour. The treatment is conducted at a temperature of 75° to 80° F. As a check, insects are exposed to the solvent alone. The insects are removed from the towers, transferred to clean petri dishes and maintained at a temperature of 80° F.  $\pm$  2° and a relative humidity of 60 percent  $\pm$  5 for post-exposure observations.

Residue Test:

Strips of aluminum foil laminated to 40-pound kraft paper, 3 by 12 inches, are treated with acetone solutions of the candidate insecticide by means of a Gardner automatic blade applicator. The paper surface of four strips each and the aluminum surface of four strips each are treated at several rates of application. Malathion is used as the standard. Twenty-eight days after the surfaces are treated, four open-end glass cylinders, 2.5 inches in diameter and 3 inches in height, are placed on each treated surface. Ten confused flour beetle adults are introduced into each cylinder, exposed to the treated surface for 24 hours, and then transferred to clean petri dishes for post-exposure observations. Knockdown is recorded 24 hours after exposure. Knockdown and dead-plus-moribund are recorded 120 hours after exposure.

Vapor Test:

Acetone solutions of the candidate insecticide are applied to the inner surface of 1-quart Mason jars. Jars are treated in quadruplicate with each insecticide at a rate of 200 mg./sq. ft. by rolling the solution evenly over the bottom and sides (up to the neck) until the solution will no longer run. The treated jars are stored open for 4 hours under a hood to allow any remaining acetone to volatilize. Ten confused flour beetle adults are place in crystallizing dishes, each covered with a canopy. The dishes are placed on lids, and the jars are inverted over the dishes. This allows the insects to be exposed to the atmosphere within the jar without coming in contact with the treated surface. Knockdown is recorded at 24 hours' exposure. Knockdown and dead-plus-moribund are recorded at 120 hours' exposure. Lindane and malathion are used as standards.

Direct Contact Test (Topical Application):

Adults are anesthetized by placing them in a Büchner funnel and introducing carbon dioxide through a tube from a pressurized tank. The insects are picked up individually by means of a suction tube and 0.5 microliter of the insecticide formulation is applied to the dorsal thoracic region by means of an ISCO microapplicator with 1/4-cc. syringe and 27-gauge needle. As a check, insects are treated in a similar manner using solvent alone. The insects are placed in clean petri dishes and maintained at a temperature of 80° F.  $\pm$  2° and a relative humidity of 60 percent  $\pm$  for postexposure observations.

Test Method - Repellents

Coleoptera

Tribolium castaneum - red flour beetle

Savannah, Georgia

Laboratory Repellency Test:

Strips of kraft paper, 4 x 16 inches, are treated with acetone solutions of the candidate repellent by means of a Gardner automatic blade applicator. Four strips each are treated at several rates of application. Papers treated with pyrethrins at 10 milligrams per square foot, piperonyl butoxide at 100 milligrams per square foot, and pyrethrins at 10 milligrams per square foot in combination with piperonyl butoxide at 100 milligrams per square foot are used as standards for comparison. For testing, a strip of treated and a strip of untreated paper are joined edge-to-edge with cellulose tape on the bottom side and the joined strips cut into two sections, each 8 inches long. The two sections are positioned so that the treated half of one will be turned to the right and the treated half of the other turned to the left to counteract any undetermined external influence on the distribution of the test insects. Two glass cylinders, 3 inches in height and 3 inches in outside diameter, are placed on the joined papers to provide test arenas of equal areas of treated and untreated paper. Ten adult flour beetles are exposed in each test arena. The average number of insects on the untreated half of the repellency arena during a 5-day period is determined initially 4 days after the papers are treated and, when warranted, 2 weeks and 1, 2, 3 months following application of the compounds. The averages are converted to express "percent repellency or attractancy" by doubling the difference between the percentage of insects counted on the untreated half and the 50-percent expected distribution if only untreated papers were used. Positive figures (+) express repellency and negative figures (-) attractancy.

PLANT PEST CONTROL DIVISION

Test Methods - Insecticides

Coleoptera

Graphognathus spp. - white-fringed beetle

Gulfport, Mississippi

Dip Test:

Mature, field-collected larvae are submerged in 1 percent acetone solutions of the candidate insecticide for 30 seconds. Preliminary tests showed that submersion in acetone for 1/2 to 4 minutes was not toxic to mature larvae. After exposure to the insecticide solution, the larvae are placed on paper toweling to remove excess liquid. The treated larvae are held in 1-ounce plastic medicine dispensers with moist soil and a small cube of Irish potato for food.

Mortality records are made at weekly intervals over a 4 week period. Tests with check mortalities in excess of 20 percent are terminated and repeated.

Candidates giving high kills are further tested by this procedure and other tests, i.e., soil tests, topical applications, etc., until the complete potential of the candidate is determined.

Hymenoptera

Solenopsis saevissima - imported fire ant

Gulfport, Mississippi

Soil Treatment Test:

Tests are conducted in small plastic flower pots which have an upright rim 16 mm. in height and 63 mm. in diameter at the top. Below the rim, the diameter narrows to 58 mm., forming a ridge 2.5 mm. wide. The pot tapers from there to 42 mm. at the bottom. There are three small holes in the bottom of the pot. A layer of plaster of paris and cement (9 to 1 ratio), about 1/4 inch thick, is poured over the bottom. This mixture seals the holes and acts as a wick to draw up moisture when the pots are set on wet peat moss. Moisture is necessary to wet the soil and maintain a high humidity in the test chamber. The cement is added to the plaster of paris to make a mixture that the ants cannot tunnel through and thereby escape.

The candidate insecticides are dissolved in acetone and diluted to a concentration which gives 1.0 p.p.m. in the soil when 5 milliliters of the solution are added to 20 grams of soil. The acetone is evaporated from the soil at 77  $\pm$  2 degrees F. under a hood. One level teaspoon of the treated soil is placed in each flower pot. Twenty worker ants from a selected field colony are placed in each pot. The sides of the pot are dusted with talc to force the ants to remain on the treated soil. A small glass disc with a diameter slightly less than the rim of the pot is placed on the ridge formed at the juncture of the rim and the slanted portion of the pot. This disc insures against escape of the ants and helps maintain high humidity in the test chamber. After 24 hours the ants are removed from the treated soil and placed in clean flower pots containing no soil. Peanut oil, soaked on cotton and placed in small vial caps, is provided for food. Counts for dead and moribund ants are made at twenty-four hour intervals for 96 hours from the beginning of the test. All tests are run in duplicate.

Insecticides are tested first at 1.0 p.p.m. in the soil. Materials giving less than 50 percent mortality after 96 hours are eliminated. Those giving more than 50 percent mortality at this level are diluted and tested at 0.1 p.p.m. The procedure is repeated, reducing concentration of the toxicant in the soil by ten-fold increments until a level is reached which gives less than 50 percent mortality after 96 hours. Materials giving 90 to 100 percent mortality at 0.1 p.p.m. in the soil are considered promising for field testing. The insecticides are rated according to their toxicity to the imported fire ant. The following are the classifications used:

- Class I      - Insecticides which give less than 50 percent mortality at 1.0 p.p.m. in the soil after 96 hours.
- Class II      - Insecticides which give more than 50 percent mortality at 1.0 p.p.m. but less than 50 percent mortality at 0.1 p.p.m. in the soil after 96 hours.
- Class III      - Insecticides which give more than 50 percent mortality at 1.0 p.p.m. and 0.1 p.p.m. but less than 50 percent mortality at 0.01 p.p.m. in the soil after 96 hours.
- Class IV      - Insecticides which give more than 50 percent mortality at 1.0, 0.1, and 0.01 p.p.m. in the soil after 96 hours.

Bait Toxicant Test:

A test chamber consisting of a small plastic flower pot with a rim 16 mm. in height and 63 mm. in diameter at the top is used. Immediately below the rim of the pot the diameter narrows to 58 mm., forming a ridge 2.5 mm. wide. The top tapers from there to 42 mm. at the bottom. The three small holes in the bottom of the pot are covered with a 1/4-inch layer of plaster of paris mixed with cement (9 to 1 ratio). The cement is added to the plaster of paris to

make a harder medium which the ants cannot tunnel through and thereby escape. The plaster of paris acts as a wick to draw up water when the pots are placed on wet peat moss and thus maintains the high humidity which is essential for survival of the ants. The tops of the pots are covered with small plate glass discs that rest on the ridge between the rim and the tapered sides.

The ants used in the tests are collected in the field in the same general locality. The colony and a portion of its mound are held in the laboratory in large galvanized tubs. Preliminary feeding tests are run during a 3-day holding period to ascertain that the colony has not been overfed or starved. Twenty worker ants are placed in each test chamber the afternoon of the day before the test begins. This permits the ants time to recover from the  $\text{CO}_2$  anesthetic and to orientate themselves to the container. The toxic bait is placed in the test chamber the next day. In preparing the toxic baits the candidate insecticides are dissolved directly in the food material; i.e., peanut oil, or 10 percent sucrose solution, depending on the solubility of the chemical. The bait is offered to the ants on cotton plugs saturated with the material and placed in small vial lids. In preliminary tests all chemicals are tested at concentrations of 1.0%, 0.1%, and 0.01%. Any materials that give complete kill at the lowest dosage are further tested until the lowest concentration which gives complete kill is determined.

The ants are allowed ad libitum feeding on the toxic bait during the first 24 hours. After this initial exposure period, the vial lids containing toxicants are removed from the cups, and the ants are kept without food for 24 hours. At the end of this period, new vial lids with peanut oil are placed in each chamber and left there for the remainder of the test. Eight knockdown and mortality counts were made at intervals of 1, 2, 3, 6, 8, 10, 13, and 14 days following exposure.

Bait toxicants are classified according to their effectiveness by the following system. Delayed toxicity is defined as less than 15% mortality after 24 hours and more than 89% mortality at the end of the test period.

Class I - Compounds that give insufficient kill at the preliminary test concentrations (less than 90% kill at the end of the test period).

Class Ia - Maximum kill 0 to 29%  
Ib - Maximum kill 30 to 59%.  
Ic - Maximum kill 60 to 89%.

Class II - Compounds that kill too fast at the higher concentrations but give insufficient kill at the lower concentration, i.e., 15% or more kill after 24 hours and 90 to 100% at the end of the test period at the higher concentrations but less than 90% kill with the lower concentrations at the end of the test period.

Class IIa - Produced fast kill at 1.0%.

IIb - Produced fast kill at 0.1% and 1.0%.

IIc - Produced fast kill at 0.01%, 0.1%, and 1.0%.

Class III - Compounds that show delayed action over a 1- to 9-fold dosage range.

Class IIIa - Delayed action occurred between 0.25% to 1.0%.

IIIb - Delayed action occurred between 0.025% to 0.1%.

IIIc - Delayed action occurred between 0.0025% to 0.01%.

Class IV - Compounds that show delayed action over a 10- to 99-fold dosage range.

\* Class V - Compounds that show delayed action over a 100-fold or greater dosage range.



